

AMENDMENTS TO THE SPECIFICATION

IN THE SPECIFICATION

On page 14, line 22, please replace the original paragraph with the following amended paragraph:

-- **Fig. 14** shows the map of the plasmid pTNS15. A non-functional restriction site is indicated with an asterisk (SEQ ID NO: 43). The AocIXbaI sequences are SEQ ID NOS: 44 and 45) --

On page 15, line 5, please replace the original paragraph with the following amended paragraph:

-- **Fig. 20** shows the map of the plasmid pTNS30. The linker sequence is SEQ ID NO: 41 --

On page 15, line 7, please replace the original paragraph with the following amended paragraph:

-- **Fig. 21** shows the map of the plasmid pTH1. The linker sequence is SEQ ID NO: 41 --

On page 15, line 9, please replace the original paragraph with the following amended paragraph:

-- **Fig. 22** shows the map of the plasmid pTH2. The linker sequence is SEQ ID NO: 40--

On page 16, line 5, please replace the original paragraph with the following amended paragraph:

-- For construction of an EGI-HFBI fusion protein, *hfbI* (SEQ ID 1) coding region (from Ser-23 to the STOP codon) and a peptide linker (Val Pro Arg Gly Ser Ser Ser Gly Thr Ala Pro Gly Gly)(SEQ ID NO: 38) preceding it was amplified with PCR using pTNS9 as a template and as a 5' primer TCG **GG CACTACGTG C CAG TAT AGC AAC GAC TAC TAC TCG CAA TGC** CTTGTTCCG CGTGGC TCTAGT TCT GGA ACC GCA (SEQ ID 2) and as a 3' primer TCG

TAC GGATCC TCA AGC ACC GAC GGC GGT (SEQ ID 3). pTNS9 has been described in detail in Example 19. The sequence in bold in the 5' primer encodes 16 C-terminal residues of EGI. The sequence in italics is a thrombin cleavage site and the underlined CACTACGTG is a DraDIII site. The underlined GGATCC in the 3' primer is a BamHI site. The 280 bp PCR fragment was purified from agarose gel and ligated to pGEM-T T/A vector (Promega) resulting in pMQ102. --

On page 31, line 13, please replace the original paragraph with the following amended paragraph:

-- A 280 bp DNA fragment containing a modified *cbh2* linker region followed by the coding region of *hfbI* from Ser-23 to the STOP codon was amplified by PCR using the plasmid pAR01 (Nakari-Setala *et al.*, 1996) as a template. The 5' primer was 5' TCT AGC AAG CTT **GGC TCT ACT TCT GGA ACC GCA CCA GGC GGC** AGC AAC GGC AAC GGC AAT GTT TGC (SEQ ID 14) and the 3' primer was 5' TCG TAC AAG CTT TCA AGC ACC GAC GGC GGT (SEQ ID 15). The sequences in bold in the 5' and 3' primers encode the modified CBHII linker (Gly Ser Ser Ser Gly Thr Ala Pro Gly Gly)(SEQ ID NO: 39) and a translational STOP, respectively, and the underlined AAGCTT in both primers is a HindIII site. The PCR fragment was purified from agarose gel, digested with HindIII and ligated to HindIII digested and SAP treated (Shrimp Alkaline Phosphatase, USB) pSP73 resulting in plasmid pTNS9. --

On page 31, line 25, please replace the original paragraph with the following amended paragraph:

-- For subsequent cloning of the modified CBHII linker-HFBI fragment to an *E. coli* expression vector, pTNS9 was digested with HindIII and the proper fragment was purified from agarose gel. This HindIII

fragment was cloned to HindIII digested and SAP treated (Shrimp Alkaline Phosphatase, USB) B599 resulting in pTNS13 (Figure 12). The *E. coli* expression vector B599 is essentially the same as the one described by Linder *et al.* (1996) except that it is missing a STOP codon at the end of the protein coding sequence. It carries the coding sequence for a fusion protein containing CBHII CBD (41 N-terminal residues of CBHII) and CBHI CBD linked together via CBHI linker region (CBHI linker and CBD are the last 57 residues in CBHI). The expression and secretion of the fusion protein in B599 is under the control of *tac* promoter and *pelB* signal sequence (Takkinen *et al.*, (1991). pTNS13 expression vector thus carries the coding region for a fusion protein of double CBD and HFBI linked in frame via the Gly-Ser-Ser-Ser-Gly-Thr-Ala-Pro-Gly-Gly (SEQ ID NO: 39) peptide. This vector also contains the *amp* gene for selection of *E. coli* transformants. pTNS13 plasmid was transformed into *E. coli* strain RV308 (*su*-, Δ *lacX74*, *gal*ISII::OP308, *strA*) and this strain was used for production of the fusion protein. --

On page 37, line 21, please replace the original paragraph with the following amended paragraph:

-- For construction of an expression cassette for production of HFBI -dCBD fusion protein under *cbhI* promoter, the protein coding region of *hfbI* was amplified with PCR using pEA10 (Nakari-Setälä *et al.* Eur. J. Biochem. (1996) 235:248-255) as a template. GGA ATT **CCG CGG ACT GCG CAT** CAT GAA GTT CTT CGC CAT CGC C (SEQ ID 26) was used as a 5' primer in the PCR and TGA ATT CCA TAT GTT *AGG TAC CAC CGG GGC CCA TGC CGG TAG AAG TAG AAG CCC CGG GAG CAC CGA CGG CGG TCT GGC AC* (SEQ ID 27) as a 3' primer. The sequence in bold in the 5' primer is 16 bp of *cbhI* promoter adjacent to translational start site of the corresponding gene and the underlined CCGCGG is a KspI site. The underlined and bold sequences

in the 3' primer are NdeI and Asp718 sites, respectively. The sequence in *Italics* in the 3' primer encodes for a Methionine-containing linker (PGASTSTGMGPGG)(SEQ ID NO: 41). The resulting fragment of 370 bp was digested with KspI and NdeI and ligated to pAMH110 (Nevalainen, K.M.H., Penttila, M.E., Harkki, A., Teeri, T.T. and Knowles, J. (1991) In *Molecular Industrial Mycology*. Eds. Leong, S.A. and Berka, R.. Marcel Dekker. New York) digested with same restriction enzymes. The resulting plasmid is pTNS29-2Asp. --

On page 38, line 24, please replace the original paragraph with the following amended paragraph:

-- pTNS30 (Figure 20) expression vector thus carries the coding region for a fusion protein consisting of HFBI and double CBD linked in frame via the Methionine linker peptide (PGASTSTGMGPGG) (SEQ ID NO: 41). Expression of the fusion protein is regulated by the *cbhI* transcriptional control sequences. The expression cassette may be released from the plasmid with EcoRI and SphI. - -

On page 39, line 5, please replace the original paragraph with the following amended paragraph:

-- An expression vector was constructed for production of a fusion protein consisting of *T. reesei* HFBI protein in the N-terminus and in the C-terminus a single chain antibody recognizing a small molecular weight derivative of diarylalkyltriazole (ENA5ScFv). Production of the fusion protein is under the *cbhI* regulatory sequences. For construction of HFBI-ENA5ScFv fusion protein, pENA5ScFv was digested with NcoI and XbaI. The fragment containing the *ena5scfv* gene and the histidine tail (6 x His) was blunt-end cloned to pTNS29 resulting in pTH1 (Figure 21). PENA5ScFv vector carries the coding region for ENA5 single chain antibody consisting of the variable domains of the heavy and light chains connected via a glycine serine linker and a 6 x

histidine tag at the C-terminal end. Transcription and secretion of the single chain antibody are under control of the *tac* promoter and *pelB* signal sequence, respectively (Takkinen *et al.*, 1991). pTNS29 vector carries the *hfbI* coding region of *T. reesei* followed by a linker sequence (ProGlyAlaSerThrSerThrGlyMetGlyProGlyGly) (SEQ ID NO: 41) under the control of *cbhI* promoter and terminator sequences. --

On page 43, line 15, please replace the original paragraph with the following amended paragraph:

--HFBI-dCBD protein produced by the strain VTT-D-99727 has a methionine (PGASTSTGMGPGG) (SEQ ID NO: 41) designed in the linker region between the HFBI and the dCBD, which would enable the recovery of the native HFBI and dCBD after chemical cleavage with CNBr. --

On page 48, line 5, please replace the original paragraph with the following amended paragraph:

-- *T. reesei* strain VTT-D-99726 (QM9414 $\Delta hfb2$) was cultivated on lactose-based medium in 15 L fermentor as described in Example 4. After cultivation, 1 l of the culture filtrate was subjected to ATPS with 5% polyoxyethylene detergent C12-18EO5. After phase separation at 30°C, detergent phase was separated from the depleted aqueous phase and analysed by SDS-PAGE. The analysed sample contained a protein which was about 7.5 kDa. In addition, some larger proteins were present. These were removed by hydrophobic interaction chromatography on a Phenyl-Sepharose FF column equilibrated with 50 mM sodium acetate - 1 M (NH₄)₂SO₄ buffer. The proteins were eluted with descending (NH₄)₂SO₄ salt gradient. Fractions containing the ca. 7.5 kDa protein were pooled and concentrated, and analysed by mass spectrometry. This revealed that the purified protein was

degraded to three peptides (2486, 2586 and 2709 Da), which were held together by disulphide linkages.

The N-terminal amino acid sequence from the 2486 Da peptide was ANAFCPEGLLYTNPLCCDLL(SEQ ID NO: 46), which on the basis of position of cysteines and on sequence comparison to known hydrophobins is typical to a hydrophobin. --

AMENDMENTS TO THE SEQUENCE LISTING

IN THE SEQUENCE LISTING

Please replace the Sequence Listing of record with the Substitute Sequence Listing enclosed herewith.